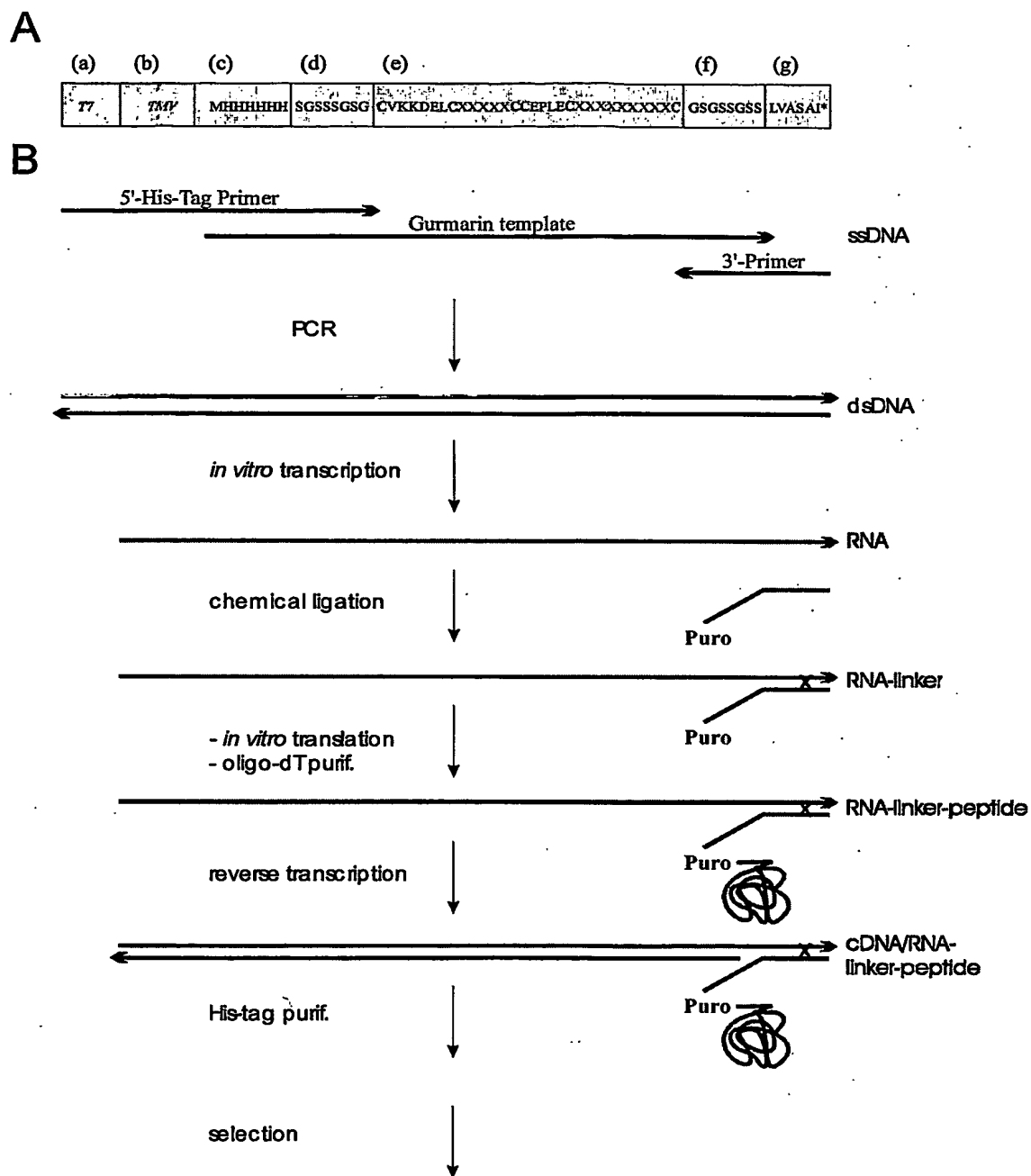
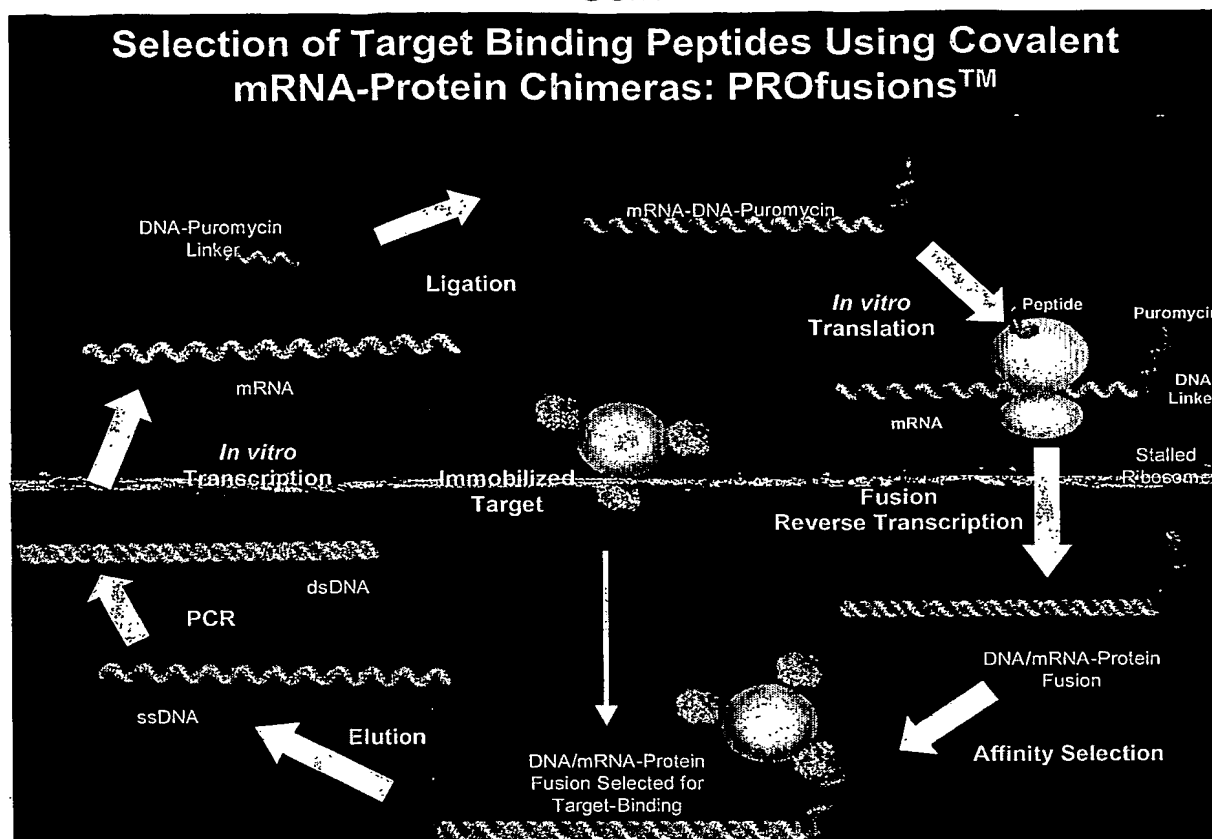


FIGURE 1

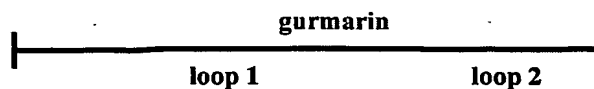


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FIGURE 2

Target: Pertussis toxin		Library: Gurmarin									
		R1	R2	R3	R4	Epoxy-PT	biot PT	biot PT	R6a	R6b	
1.	PCR										
1.1.	Analytical RT-PCR										
	specific signal after x rounds of PCR		18	24	24	24			24	15	
	control signal after y rounds of PCR		21	27	24	27			27	24	
1.2.	Preparative PCR										
	done with z rounds of PCR		36	30	30	32			30	30	
2.	In vitro transcription										
	scale		2x 500 µl	1x 500 µl	1x 500 µl	1x 500 µl			1x 500 µl	1x 500 µl	
	DNase digestion done		no	yes	yes	yes			yes	yes	
3.	NAP5 gel filtration										
	Inp/E1/E2/E3		500/300/400/200	500/300/400/200	500/300/400/200	500/300/400/200			500/300/400/200	500/300/400/200	
	yield purified RNA in pooled E1 (OD260)		20 nmol	13 nmol	12.7 nmol	11.6 nmol			16.3 nmol	17.8 nmol	
	remaining material					6.6 nmol					
4.	Linker coupling										
	Linker type		PEG2A18						PEG2	PEG2	
	input		6 nmol	5 nmol	5 nmol	5 nmol			5 nmol	5 nmol	
	coupling efficiency		70%	70%	70%	70%			70%	70%	
	yield linker coupled RNA		4.2 nmol	3.5 nmol	3.5 nmol	3.5 nmol			3.5 nmol	3.5 nmol	
5.	In vitro Translation and PROFUSION™										
	input		4.2 nmol	3.5 nmol	3 nmol	3 nmol			3 nmol	3 nmol	
	RNA / 200 µl lysate		260 pmol	290 pmol	250 pmol	250 pmol			250 pmol	250 pmol	
	salt incubation at -20°C		over night	over night	over night	over night			over night	over night	
6.	Oligo(dT) purification										
	purified on x columns		7	4	4	4			4	4	
	efficiency		4.5%	4.4%	2.7%	7.2%			3.3%	2.7%	
	yield		187 pmol	158 pmol	81.1 pmol	217 pmol			97.6 pmol	81.1 pmol	
7.	Reverse transcription										
	conditions		1 mM DTT	1 mM DTT	1 mM DTT	1 mM DTT			1 mM DTT	1 mM DTT	
	input		168 pmol (3/4)	72.6 pmol (1/2)	50.4 pmol (3/4)	50 pmol			40 pmol	50 pmol	
8.	His-Tag purification										
	input		168 pmol	90.75 pmol (72 pmol + 18 pmol dhne RT)	61.9 pmol (3/4 RT + 1/4 Odt)	45 pmol RT + 27.6 pmol RNA			35 pmol RT + 15 pmol	43 pmol RT + 19 pmol	
	efficiency		34%	78%	43%	41%			36%	43%	
	yield		66.6 pmol	59.2 pmol	26.4 pmol	27.6 pmol			18 pmol	26.4 pmol	
	in volume of		450 µl	450 µl	450 µl	450 µl			450 µl	450 µl	
9.	selection										
	selection volume		1 ml	1 ml	1 ml	1 ml			1 ml	1 ml	
	input		18 pmol	5 pmol	5 pmol	5 pmol			5 pmol	5 pmol	
	final concentration of Imidazol		45 mM	6 mM	6 mM	11.4 mM			18.8 mM	21.5 mM	
	preclear		3x 100 µl HBS blocked streptavidin beads	3x 100 µl HBS blocked streptavidin beads	3x 100 µl HBS blocked, biotin saturated streptavidin beads	2x 100 µl HBS blocked epoxy beads			3x 100 µl HBS blocked, biotin saturated streptavidin beads	3x 100 µl HBS blocked, biotin saturated streptavidin beads	
	first preclear binding		0%	background	background	23/24 dpm			37/33/34 dpm	72/30/37 dpm	
	beads saturated with a pulis of biotin		yes	yes	yes	no			yes	yes	
	effective concentration of PT		100 nM (50 nM)	100 nM (50 nM)	100 nM (50 nM)	50 nM			100 nM (50 nM)	100 nM (50 nM)	
	conditions					90 min at RT			90 min at RT	90 min at RT	
	specific binding %				0.2%	1.0%			9.4%	11.6%	

FIGURE 3



194227	M	H	H	H	H	H	S	G	S	S	G	S	G	C	V	K	K	D	E	L	C	A	G	S	V	G	H	C	C	E	P	L	E	C	L	R	R	F	L	N	L	R	W	C	G	S	G	S	S	S	S	L	V	A	S	A	I		
194238	M	H	H	H	H	H	S	G	S	S	G	S	G	C	V	K	K	D	E	L	C	I	V	M	R	A	S	C	C	E	P	L	E	C	L	R	R	F	L	N	L	R	W	C	G	S	G	S	S	S	S	L	V	A	S	A	I		
194239	M	H	H	H	H	H	S	G	S	S	G	S	G	C	V	K	K	D	E	L	C	K	A	F	R	A	S	C	C	E	P	L	E	C	L	R	K	W	L	K	H	M	F	C	G	S	G	S	S	S	S	L	V	A	S	A	I		
194251	M	H	H	H	H	H	S	G	S	S	G	S	G	C	V	K	K	D	E	L	C	L	R	S	S	I	D	C	C	E	P	L	E	C	L	Y	K	M	Q	R	R	L	C	G	S	G	S	S	S	S	L	V	A	S	A	I			
194210	M	H	H	H	H	H	S	G	S	S	G	S	G	C	V	K	K	D	E	L	C	W	P	R	R	H	K	C	C	E	P	L	E	C	L	E	M	L	E	R	R	K	C	G	S	G	S	S	S	S	L	V	A	S	A	I			
194261	M	H	H	H	H	H	S	G	S	S	G	S	G	C	V	K	K	D	E	L	C	M	S	M	A	C	V	C	C	E	P	L	E	C	L	K	Y	H	G	Y	F	W	L	-	C	G	S	A	V	L	G	-	P	S	S	I	R	Y	L
194214	M	H	H	H	H	H	S	G	S	S	G	S	G	C	V	K	K	D	E	L	C	A	V	W	Y	D	R	C	C	E	P	L	E	C	L	T	Y	Q	S	G	Y	Y	W	L	C	G	S	G	S	S	S	S	L	V	A	S	A	I	
194226	M	H	H	H	H	H	S	G	S	S	G	S	G	C	V	K	K	D	E	L	C	G	A	P	W	Y	W	R	C	C	E	P	L	E	C	L	V	T	Y	S	G	Y	Y	S	C	G	S	S	S	S	S	L	V	A	S	A	I		
194259	M	H	H	H	H	H	S	G	S	S	G	S	G	C	V	K	K	D	E	L	C	A	R	W	D	L	V	C	C	E	P	L	E	C	L	I	Y	T	S	E	L	Y	A	T	C	G	S	G	S	S	G	-	-	-	-	-	-		
194297	M	H	H	H	H	H	S	G	S	S	G	S	G	C	V	K	K	D	E	L	C	Y	F	Y	F	P	N	C	C	E	P	L	E	C	L	R	W	V	N	D	N	Y	G	W	C	G	S	G	S	S	S	S	L	V	A	S	A	I	
194330	M	H	H	H	H	H	S	G	S	S	G	S	G	C	V	K	K	D	E	L	C	M	S	M	A	C	V	C	C	E	P	L	E	C	L	K	Y	H	G	Y	F	W	L	-	C	G	S	G	S	S	S	S	L	V	A	S	A	I	
194479	M	H	H	H	H	H	S	G	S	S	G	S	G	C	V	K	K	D	E	L	C	T	T	A	S	M	C	S	C	C	E	P	L	E	C	K	W	T	N	E	H	F	G	T	C	G	S	G	S	S	S	S	L	V	A	S	A	I	
194551	M	H	H	H	H	H	S	G	S	S	G	S	G	C	V	K	K	D	E	L	C	S	Q	S	V	P	M	C	C	E	P	L	E	C	K	W	F	N	E	Y	G	I	C	G	S	G	S	S	S	S	L	V	A	S	A	I			
194533	M	H	H	H	H	H	S	G	S	S	G	S	G	C	V	K	K	D	E	L	C	A</																																					

FIGURE 4

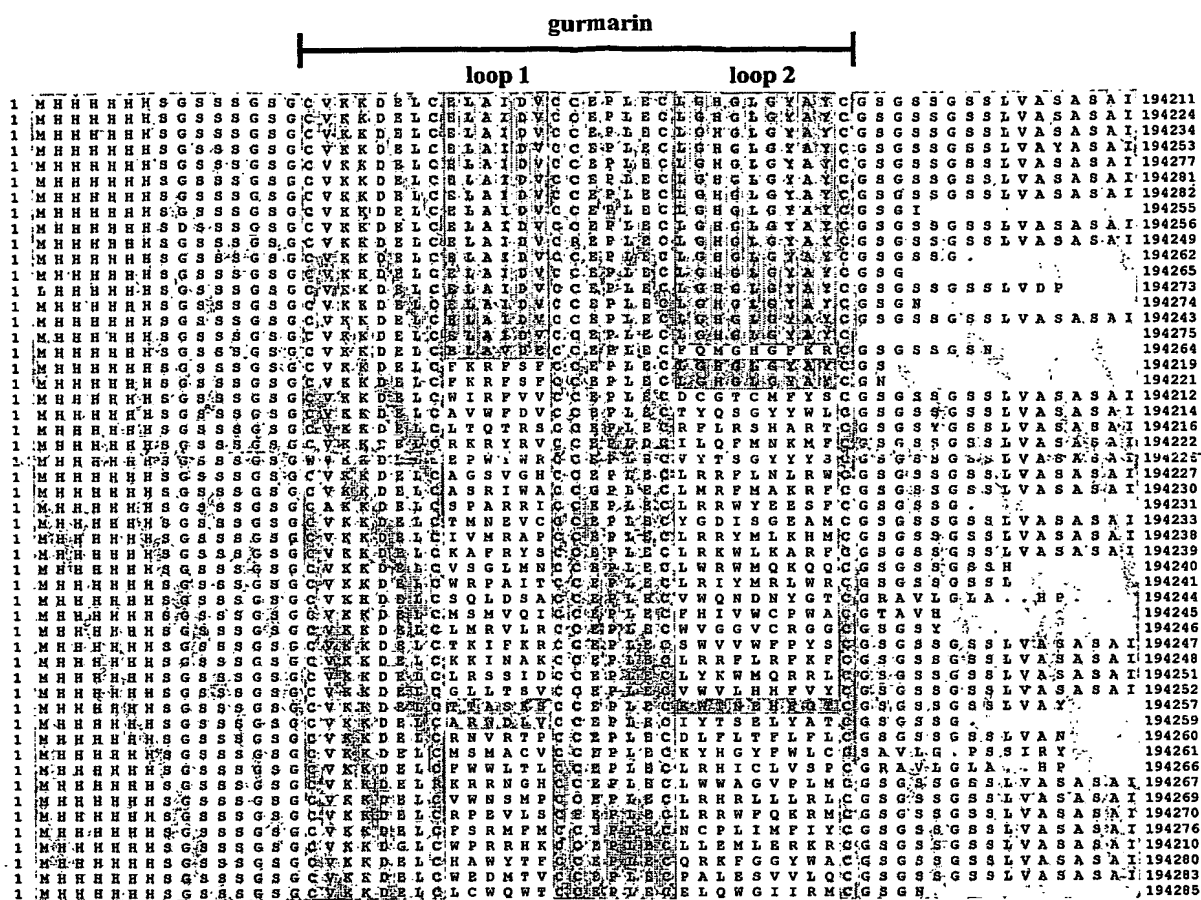




FIGURE 6

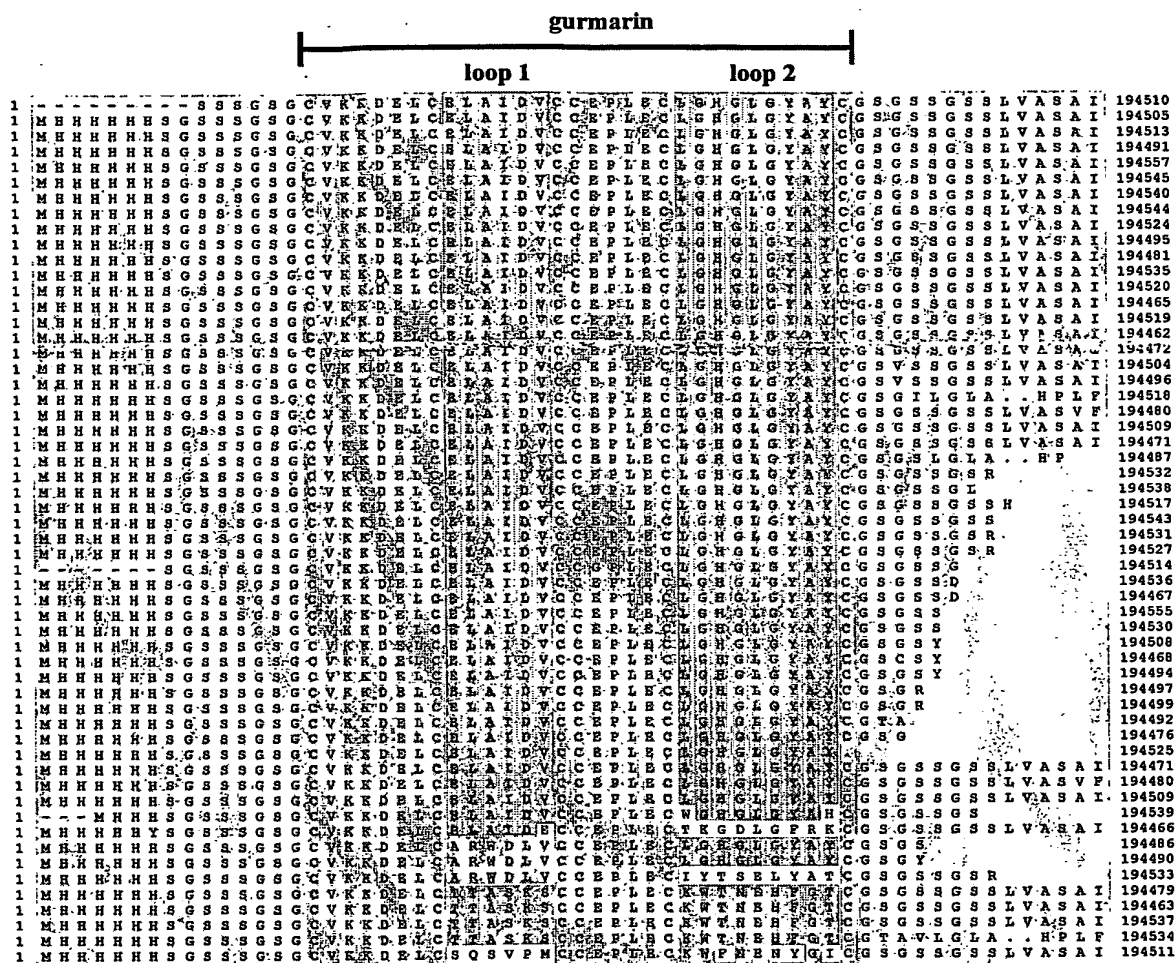


FIGURE 8

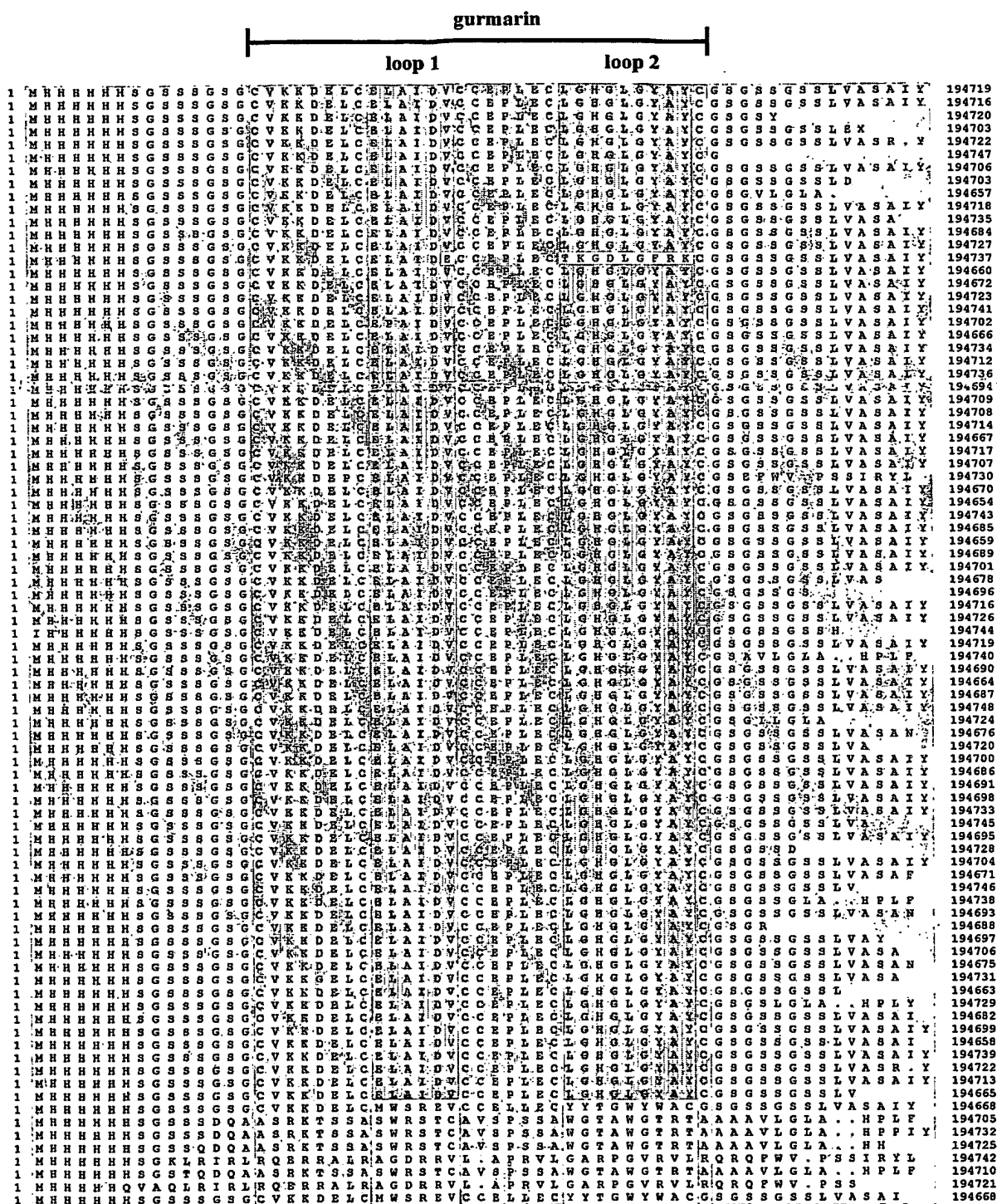


Table 2: selection overview of PP26 selection against immobilized PT

Target: Pertussis toxin		Library: PP26				epoxy bead imm. PT	biot PT	biot PT	biot PT
		R1	R2	R3	R4	R5a	R5b	R6a	R6b
1. PCR									
1.1. Analytical RT-PCR									
specific signal after x rounds of PCR			18	18	24	24			
control signal after y rounds of PCR			21	24	24	24			
1.2. Preparative PCR									
done with z rounds of PCR			38	30	30	32			
quality				single band	single band	single band			
2. <i>In vitro</i> transcription									
scale			2x 500 µ	1x 500 µ	1x 500 µ	1x 500 µ			
DNase digestion done			no	no	yes	yes			
quality									
3. NAPS gelification									
ImP/IE2/E3									
yield purified RNA in pooled E2 (OD280)			500/500/400/200	16 nmol	500/300/400/200	500/300/400/200			
4. Linker coupling									
Linker type			PEG2	PEG2	PEG2	PEG2			
input			6 nmol	4.5 nmol	5 nmol	5 nmol			
coupling efficiency			70%	70%	70%	70%			
yield linker coupled RNA			4.2 nmol	3.15 nmol	3.5 nmol	3.5 nmol			
quality									
5. <i>In vitro</i> translation and Fuesagen formation									
input			4.2 nmol	3.15 nmol	3 nmol	3 nmol			
RNA/200 µ			280 pmol	260 pmol	250 pmol	250 pmol			
cell incubation at -20°C			over night	over night	over night	over night			
6. Oligo(dT) purification									
purified on x columns			?	4	4	4			
efficiency			8%	2.1%	1.89%	58.7 pmol			
yield			88 pmol	56.7 pmol	64.9 pmol	175 pmol			
7. Reverse transcription									
conditions			1 mM DTT	1 mM DTT	1 mM DTT	1 mM DTT			
input			88 pmol (3/4)	42.5 pmol (3/4)	40.3 pmol (3/4)	50 pmol			
efficiency of RT									
yield of RT-RNA									
portion of reverse transcribed Fuesagens									
8. His-tag purification on Ni-NTA agarose									
conditions: endconcentration of DTT from RT			0.75 mM	0.7 mM	0.52 mM	0.5 mM			
input			88 pmol	42.5 pmol (RT Ansatz, ohne nickel-RT)	47 pmol (3/4 RT + 1/4 Odt)	45 pmol RT + 21 pmol RNA			
efficiency			34%	25%	30.6%	35%			
yield			55 pmol (100%)	18.7 pmol	12.2 pmol	27.8 pmol			
in volume of			450 µ	300 µ	450 µ	450 µ			
9. selection									
selection volume		1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
input		18 pmol	5 pmol	5.3 pmol	5 pmol	5 pmol	5 pmol	5 pmol	5 pmol
final concentration of imidazol		25 mM	18 mM	19.5 mM	6 mM	12.3 mM	3x 100 µM blocked, biotin streptavidin beads	3x 100 µM blocked, biotin streptavidin beads	3x 100 µM blocked, biotin streptavidin beads
preclear		5x 100 µM streptavidin beads	5x 100 µM streptavidin beads	3x 100 µM epoxy beads		2x 100 µM blocked epoxy beads	2x 100 µM blocked epoxy beads	2x 100 µM blocked, biotin streptavidin beads	2x 100 µM blocked, biotin streptavidin beads
first pre-clear binding %		0%	0.5%	background	0.5%	42 dpm	0.3%	278/214/208 dpm	7230/37 dpm
selection on biotinylated Pertussis toxin (100%)		serie 1	serie 1	serie 1	serie 1	serie 2	serie 1	serie 1	serie 1
Epoxy-bead coupled PT									
PT-beads saturated with a pulse of biotin		yes	yes	yes	yes	no	yes	yes	yes
effective concentration of PT		50 nM (100%)	50 nM (100%)	50 nM (100%)	50 nM (100%)	50 nM	100 nM (50 nM)	100 nM (50 nM)	100 nM (50 nM)
conditions		over night at 4°C	60 min at RT	90 min at RT	60 min at RT	90 min at RT	80 min at RT	80 min at RT	80 min at RT
specific binding %					1.2%	0.5%	2.8%	2.8%	3.5%

197588 MGRGSHHHHHHARS NVIPLNEVWYDTGWDREHRSRLSTD DDANAPKASAI
197947 MGRGSHHHHHHARS VGTIIRIAQDTEHYRNVRKLSQYER DANAPKASAI

198000 MGRGSHHHHHHARS WRDTRKLLHMRHYFPLAIDSYWDHTLR DANAPKASAI
197983 MGRGSHHHHHHARS ETSMDGERTLRDDELTATTKTSSEPF DANAPKASAI
197998 MGRGSHHHHHHARS SPLWYHNCWD TICLADWLKDRPHGVY DANAPKASA

197836 MGRGSHHHHHHARS [REDACTED] DANAPKASAI
197552 MGRGSHHHHHHARS [REDACTED] DANAPKASAI

197596 MGRGSHHHHHHARS TMTNRMDDIQRMTNHVKRDS SPGSI DANAPKASAI

197569 MGRGSHHHHHHARS DWELSPPHVAITTRHLINCTDGP LLRDANAPKASAI
197536 MGRGSHHHHHHARS LNGESTSNI LTTSRKVTEW TGYTASVDANAPKASAI

197611 MGRGSHHHHHHARS QVTWHLADTVTTKNRKCTDSYIGWNXANAPKASAI
197530 MGRGSHHHHHHARS IIVIHNAIQHTPHQVSIWC PPKHN RDANAPKASAI
197557 MGRGSHHHHHHARS SHCRHRNCHTITRGNMRIETFPNNIRK DANAPKASAI

197797 MGRGSHHHHHHARS SWGLSGTQTWKITKLATRLHHPEFETNDANAPKASAI

197888 MGRGSHHHHHHARS WRWHNWGLSDTVASHPDASNSLNMMY DANAPKASAN
197897 MGRGSHHHHHHLDLWGPPSGSPRTRSTTGTSSTTSSPSTPGTITLRRHPH
197825 MGRGSHHHHHHARS SWQPEVKMSSLVDTSQTVGA AVETRRTT DANAPKASA

FIGURE 9

FIGURE 10

[illegible]

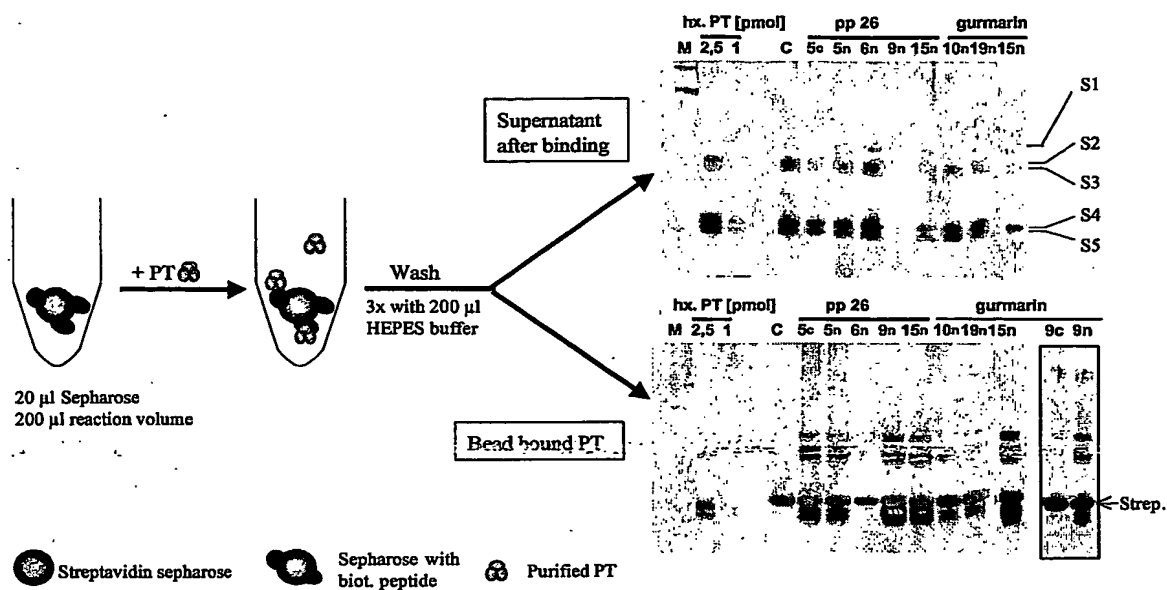
FIGURE 12

[illegible]

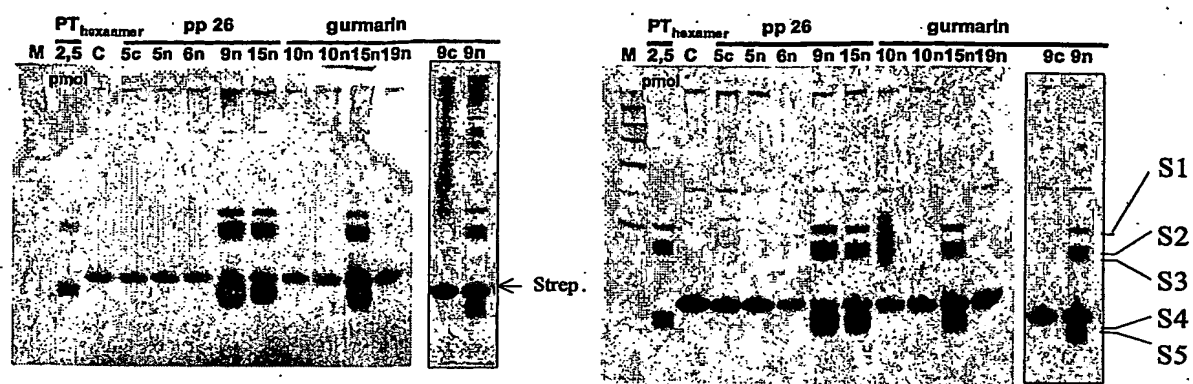
FIGURE 14

1	M	G	R	G	S	-	H	H	H	H	H	H	A	R	S	N	V	I	P	L	H	E	V	W	Y	D	T	G	W	D	R	P	H	R	S	R	L	S	I	D	O	D	A	N	A	P	K	A	S	A	I		197791
1	M	G	R	G	S	-	H	H	H	H	H	H	A	R	S	N	V	I	P	L	H	E	V	W	Y	D	T	G	W	D	R	P	H	R	S	R	L	S	I	D	O	D	A	N	A	P	K	A	S	A	I		197796
1	M	G	R	G	S	-	H	H	H	H	H	H	A	R	S	N	V	I	P	L	H	E	V	W	Y	D	T	G	W	D	R	P	H	R	S	R	L	S	I	D	O	D	A	N	A	P	K	A	S	A	I		197798
1	M	G	R	G	S	-	H	H	H	H	H	H	A	R	S	N	V	I	P	L	H	E	V	W	Y	D	T	G	W	D	R	P	H	R	S	R	L	S	I	D	O	D	A	N	A	P	K	A	S	A	I		197994
1	M	G	R	G	S	-	H	H	H	H	H	H	A	R	S	N	V	I	P	L	H	E	V	W	Y	D	T	G	W	D	R	P	H	R	S	R	L	S	I	D	O	D	A	N	A	P	K	A	S	A	I		198005
1	M	G	R	G	S	-	H	H	H	H	H	H	A	R	S	N	V	I	P	L	H	E	V	W	Y	D	T	G	W	D	R	P	H	R	S	R	L	S	I	D	O	D	A	N	A	P	K	A	S	A	I		197997
1	M	G	R	G	S	-	H	H	H	H	H	H	A	R	S	N	V	I	P	L	H	E	V	W	Y	D	T	G	W	D	R	P	H	R	S	R	L	S	I	D	O	D	A	N	A	P	K	A	S	A	I		198003
1	M	G	R	G	S	-	H	H	H	H	H	H	A	R	S	N	V	I	P	L	H	E	V	W	Y	D	T	G	W	D	R	P	H	R	S	R	L	S	I	D	O	D	A	N	A	P	K	A	S	A	I		198004
1	M	G	R	G	S	-	H	H	H	H	H	H	A	R	S	N	V	I	P	L	H	E	V	W	Y	D	T	G	W	D	R	P	H	R	S	R	L	S	I	D	O	D	A	N	A	P	K	A	S	A	I		197922
1	M	G	R	G	S	-	H	H	H	H	H	H	A	R	S	N	V	I	P	L	H	E	V	W	Y	D	T	G	W	D	R	P	H	R	S	R	L	S	I	D	O	D	A	N	A	P	K	A	S	A	I		197921
1	M	G	R	G	S	-	H	H	H	H	H	H	A	R	S	N	V	I	P	L	H	E	V	W	Y	D	T	G	W	D	R	P	H	R	S	R	L	S	I	D	O	D	A	N	A	P	K	A	S	A	I		197964
1	M	G	R	G	S	-	H	H	H	H	H	H	A	R	S	N	V	I	P	L	H	E	V	W	Y	D	T	G	W	D	R	P	H	R	S	R	L	S	I	D	O	D	A	N	A	P	K	A	S	A	I		197945
1	M	G	R	G	S	-	H	H	H	H	H	H	A	R	S	N	V	I	P	L	H	E	V	W	Y	D	T	G	W	D	R	P	H	R	S	R	L	S	I	D	O	D	A	N	A	P	K	A	S	A	I		197948
1	M	G	R	G	S	-	H	H	H	H	H	H	A	R	S	N	V	I	P	L	H	E	V	W	Y	D	T	G	W	D	R	P	H	R	S	R	L	S	I	D	O	D	A	N	A	P	K	A	S	A	I		197953
1	M	G	R	G	S	-	H	H	H	H	H	H	A	R	S	N	V	I	P	L	H																																

FIGURE 15



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FIGURE 16

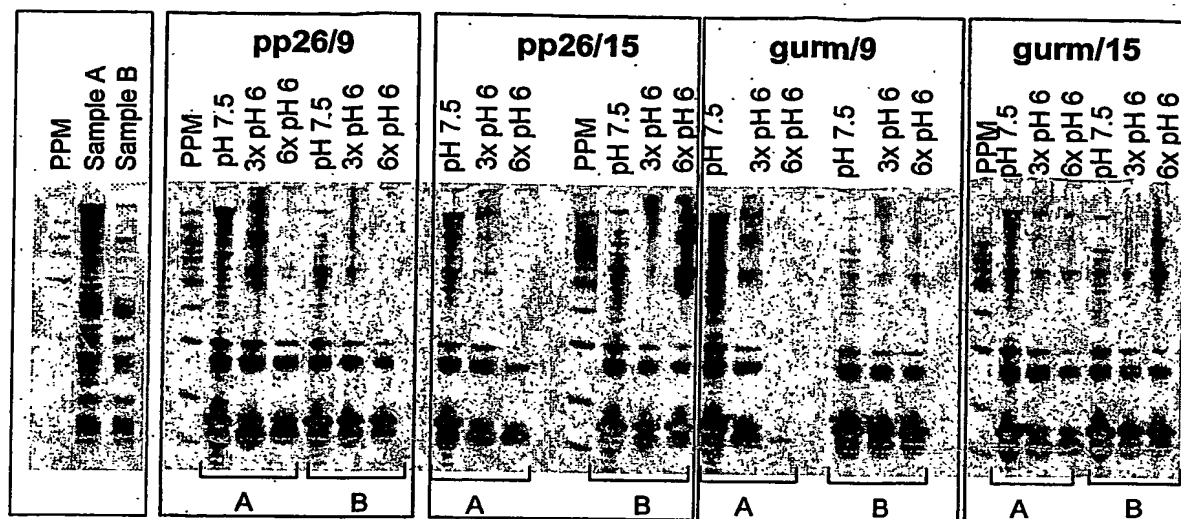
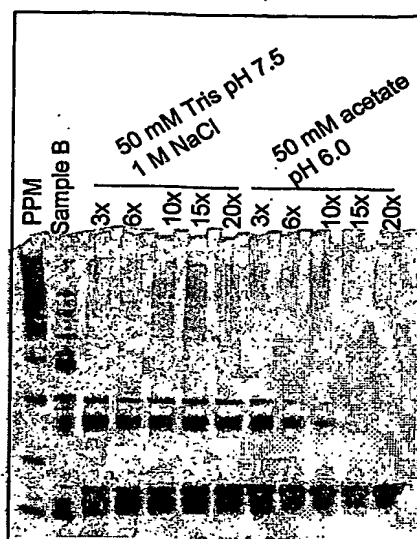
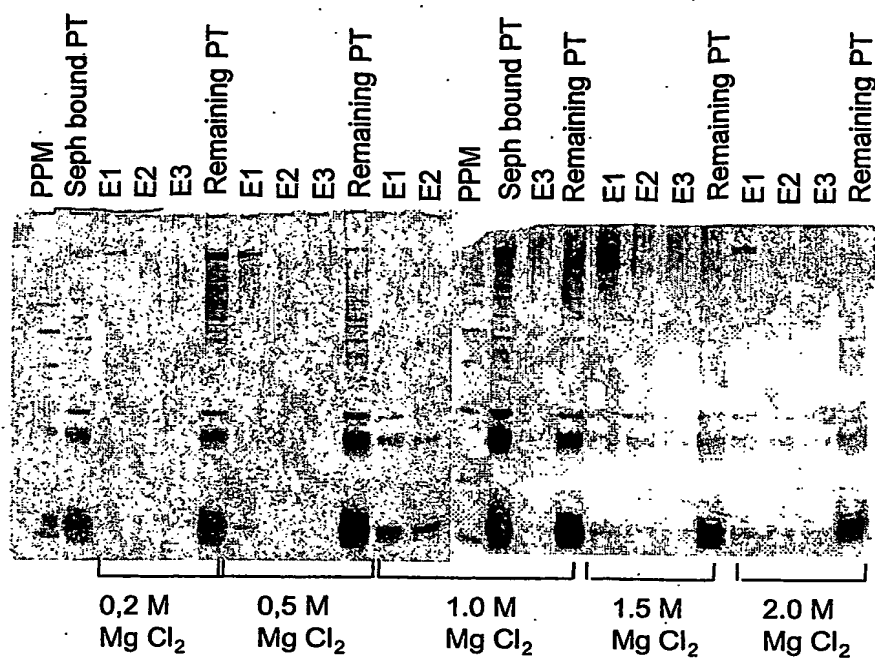
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FIGURE 17

FIGURE 18.

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Gurmarin/9

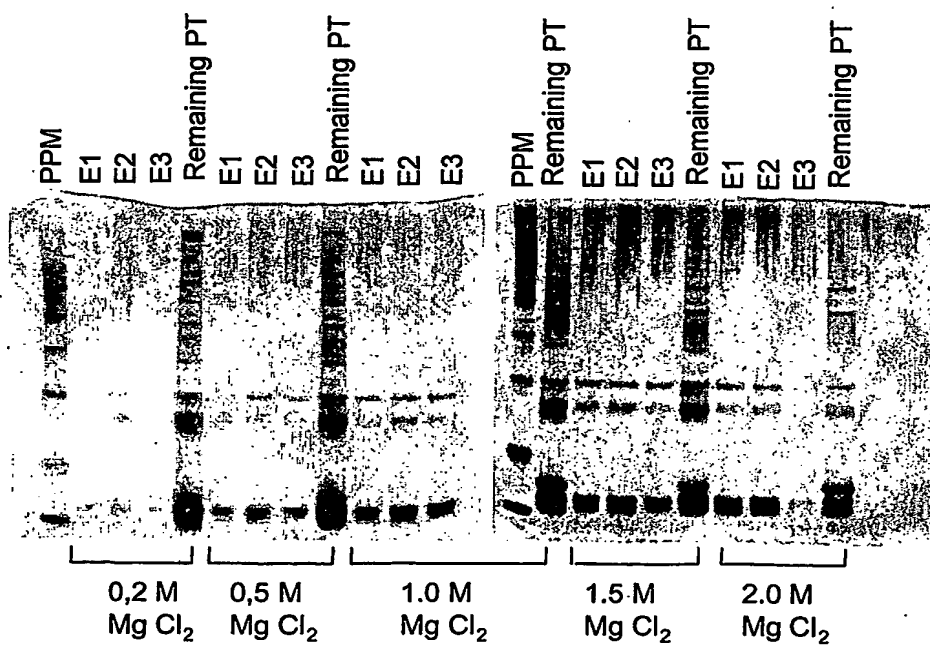


FIGURE 19

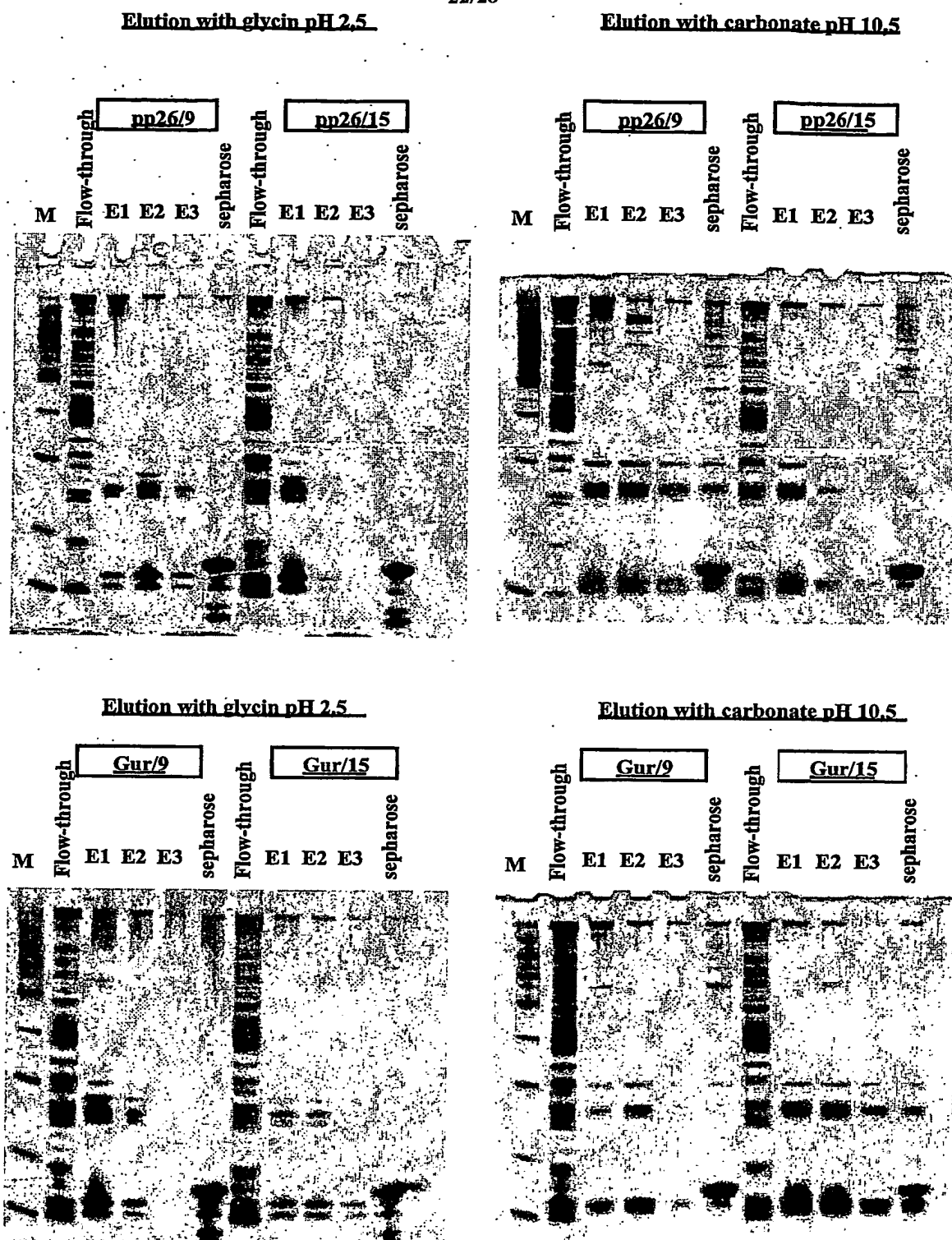
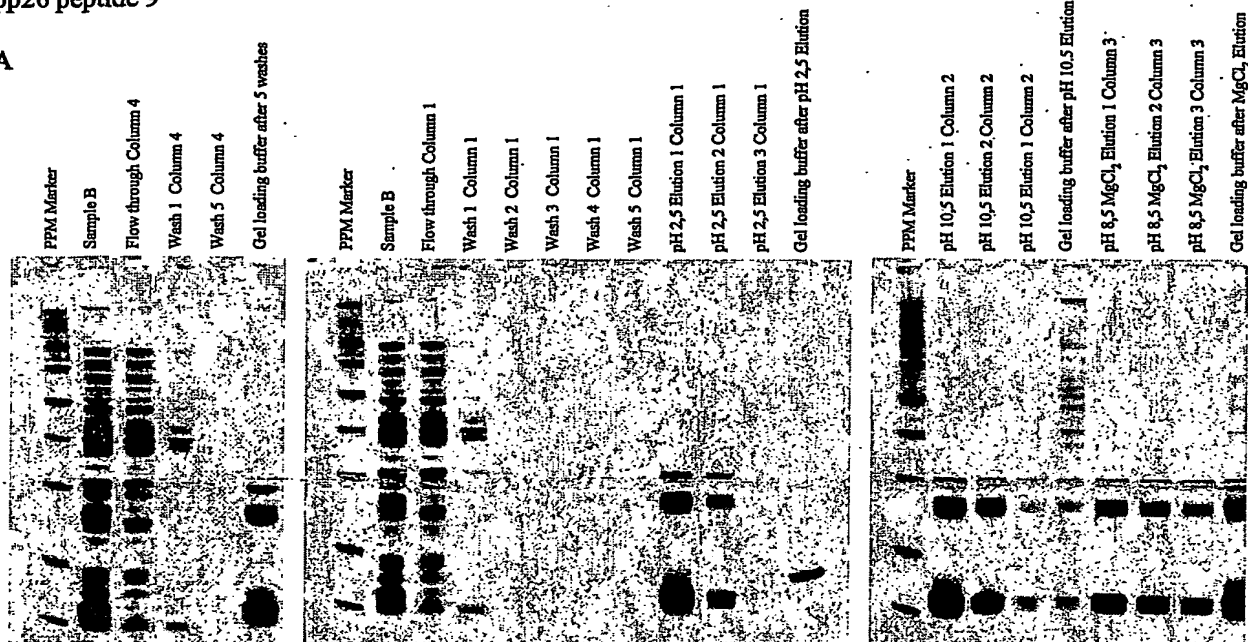


FIGURE 20

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A



B

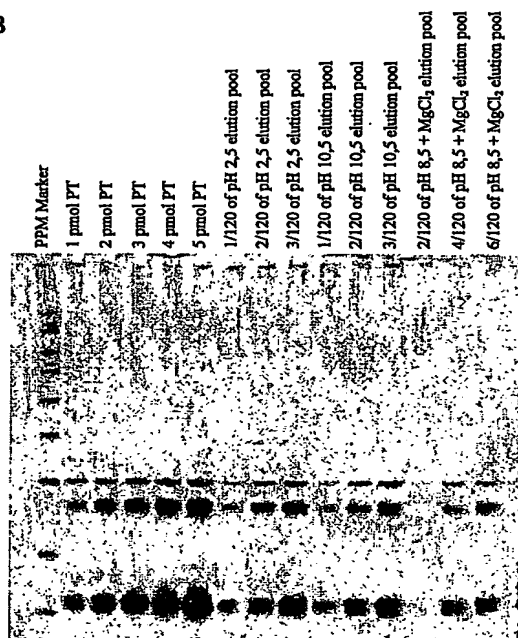


FIGURE 21

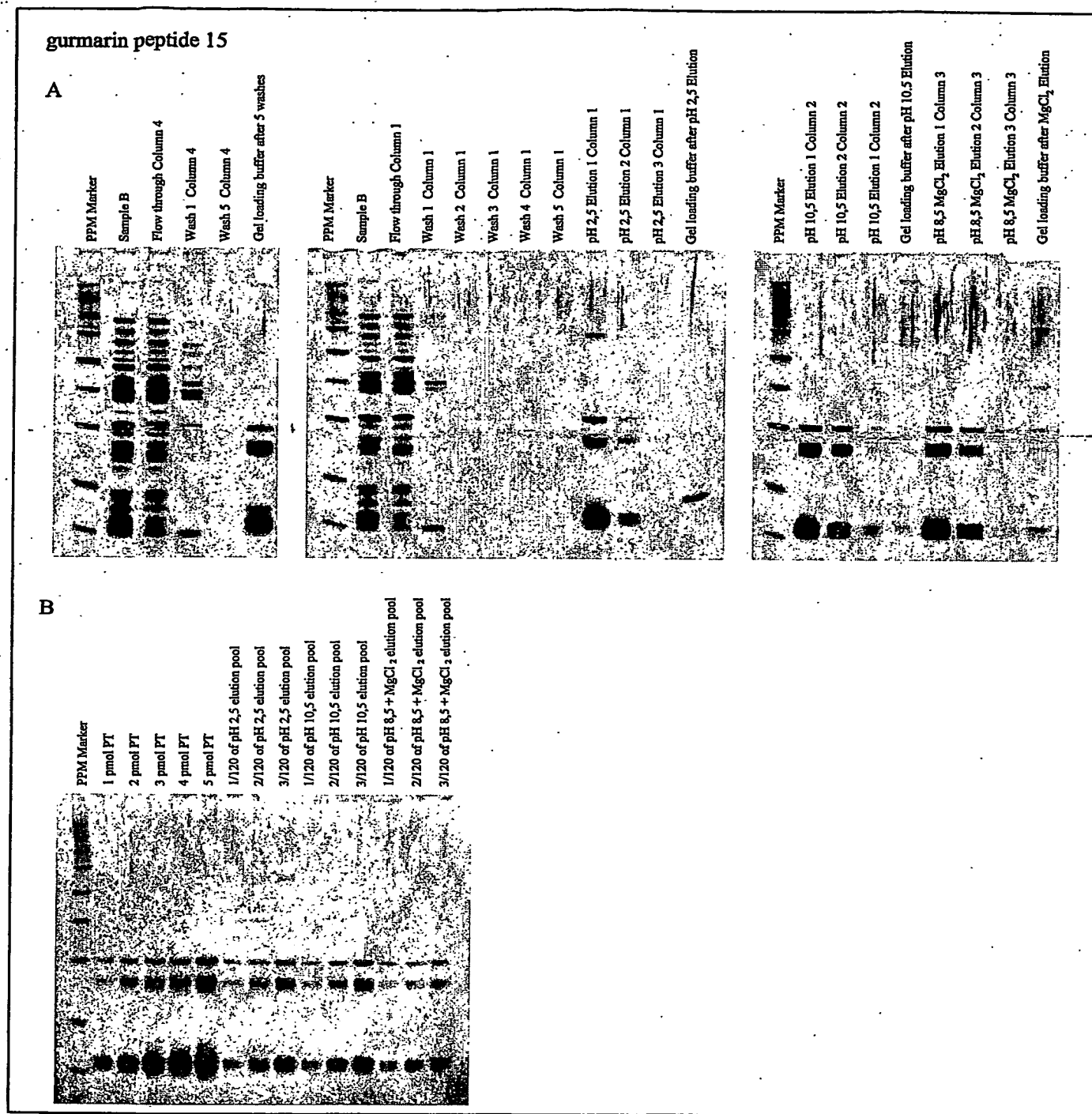
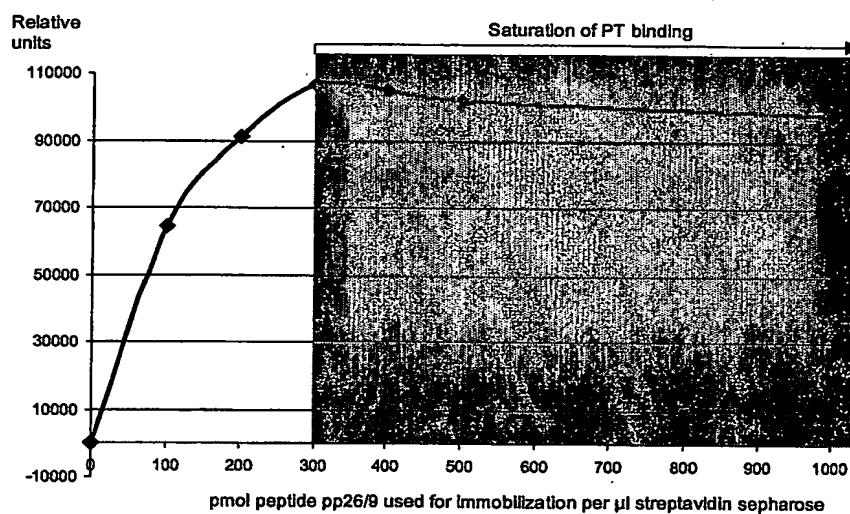
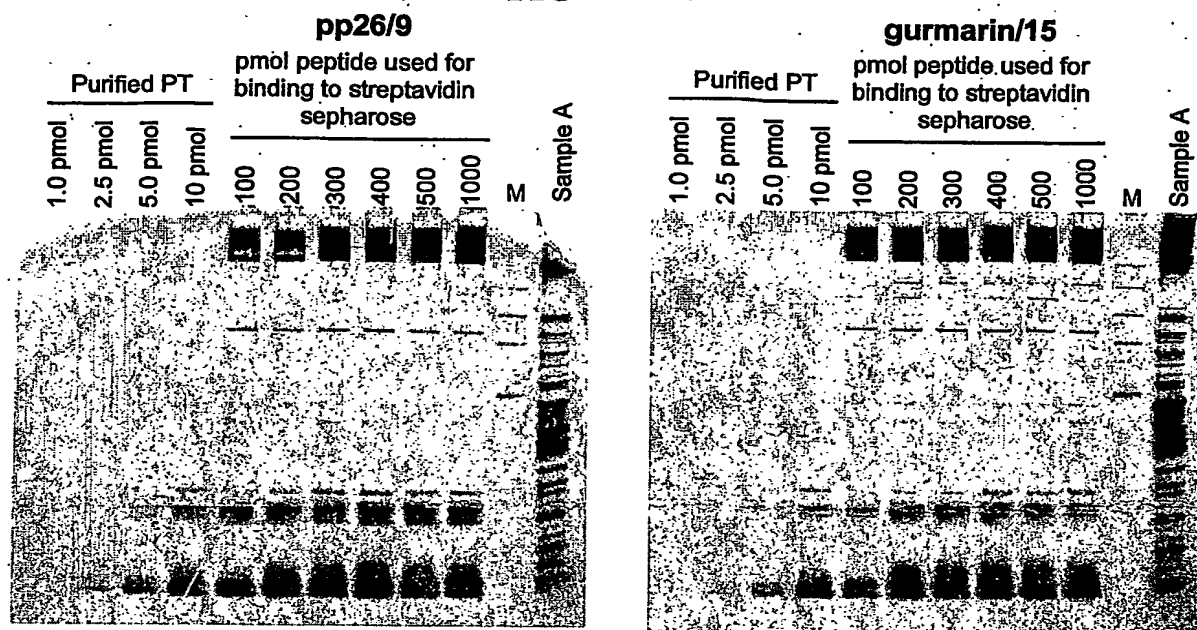


FIGURE 22

25/28
FIGURE 23

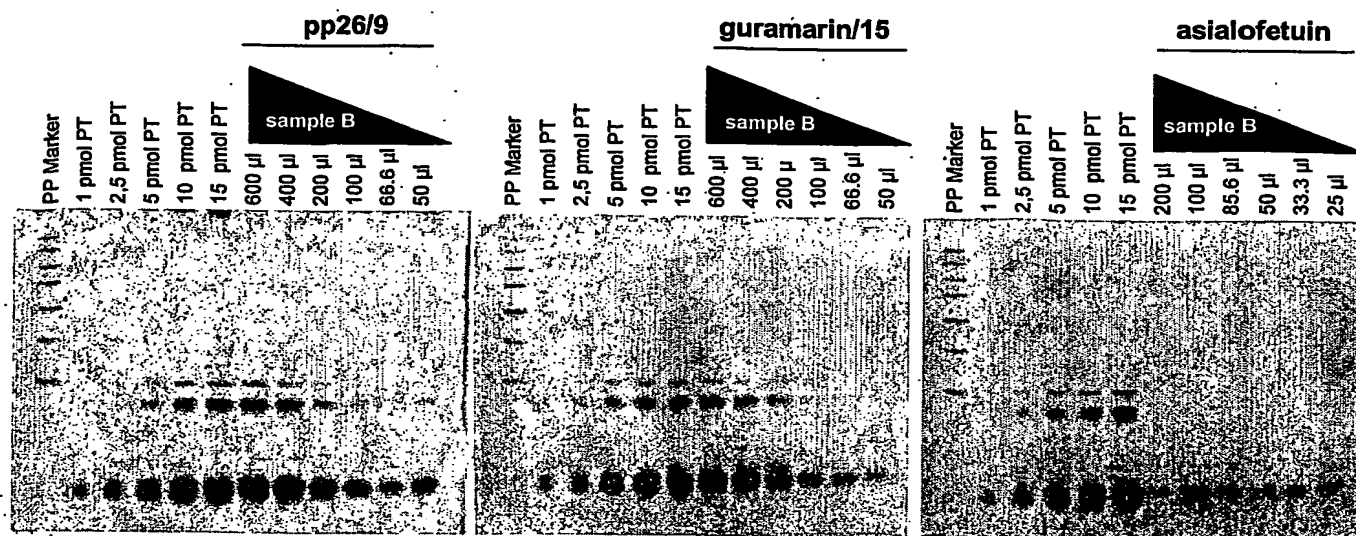


FIGURE 24

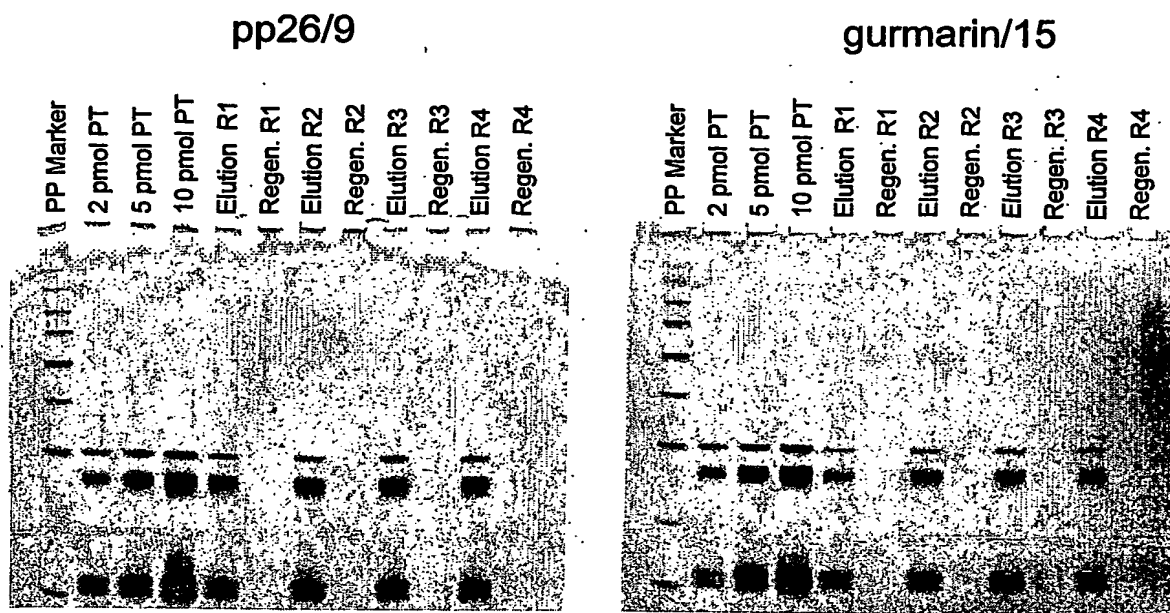


FIGURE 25

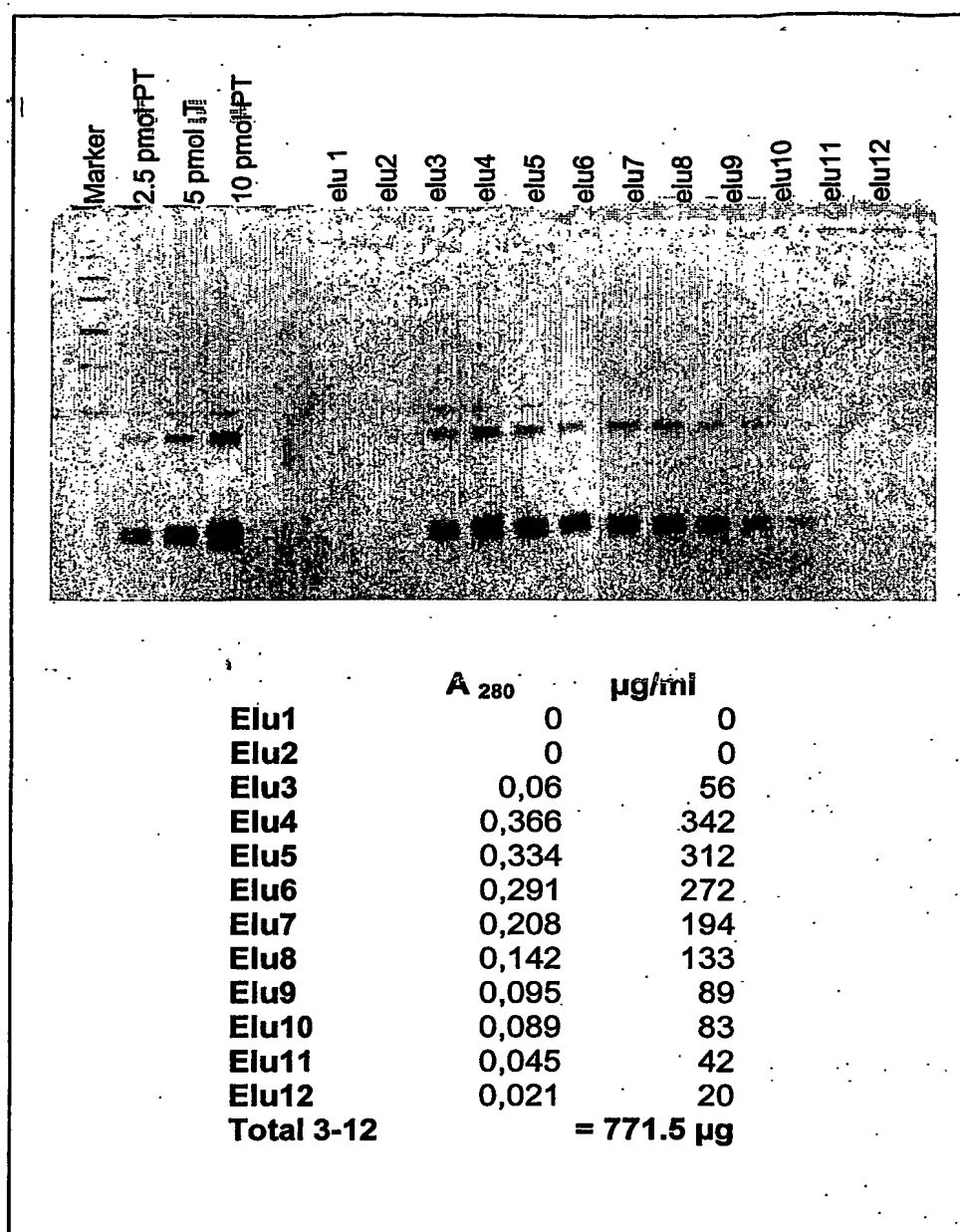


FIGURE 26

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